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Adrenoceptor functions in the cat choledochoduodenal junction *in vitro*

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Summary

1. The effects of α- and β-adrenoceptor stimulating agents were investigated on three different kinds of preparation of the isolated sphincter of Oddi and on the duodenum of the cat.

2. Adrenaline ($1{\cdot}5 \times 10^{-7}$M–$6{\cdot}3 \times 10^{-7}$M), noradrenaline ($1{\cdot}6 \times 10^{-7}$M–$6{\cdot}3 \times 10^{-7}$M), and tyramine ($2{\cdot}9 \times 10^{-6}$M–$5{\cdot}8 \times 10^{-6}$M) increased the activity and tonus of the sphincter musculature and decreased duodenal activity and tone. The effect on the sphincter resulted in increased resistance to flow through the sphincter. The excitatory effects on the sphincter were blocked by phenoxybenzamine ($1{\cdot}7 \times 10^{-8}$M–$1{\cdot}7 \times 10^{-7}$M).

3. No effect was produced by tyramine in concentrations up to $4{\cdot}6 \times 10^{-5}$M on sphincters taken from reserpinized cats. It is suggested that the cat sphincter of Oddi contains adrenergic nerves of functional importance.

4. Isoprenaline ($1{\cdot}9 \times 10^{-8}$M–$4{\cdot}7 \times 10^{-7}$M) and terbutaline ($3{\cdot}5 \times 10^{-7}$M–$8{\cdot}8 \times 10^{-6}$M) decreased spontaneous activity and tonus of the sphincter, and diminished resistance to flow through the sphincter. Both agents decreased spontaneous activity and tonus of the duodenum. On a molar basis, isoprenaline was 2–18 times more active than terbutaline on the sphincter and 35–90 times more active on the duodenum. The effects of isoprenaline and terbutaline were blocked by propranolol ($3{\cdot}9 \times 10^{-7}$M).

5. It is concluded that the cat sphincter of Oddi contains α-adrenoceptors active in contraction of the sphincter, and β-adrenoceptors active in its relaxation. The β-adrenoceptors of the sphincter differ from those in the duodenum; it is suggested that they belong to the β_2-group (according to Lands' classification).

6. The automaticity of the isolated sphincter of Oddi resembled the sphincter activity recorded *in vivo* and is probably myogenic in nature, as it resisted treatment with phenoxybenzamine ($1{\cdot}7 \times 10^{-8}$M–$1{\cdot}7 \times 10^{-7}$M), atropine ($1{\cdot}4 \times 10^{-6}$M–$5{\cdot}8 \times 10^{-6}$M), hexamethonium ($1{\cdot}4 \times 10^{-5}$M–$1{\cdot}1 \times 10^{-4}$M) and tetrodotoxin (1 μg/ml). The activity of the sphincter has no propulsive function but prevents passage of fluid through the sphincter.

Introduction

Early work (Kitakoji, 1930) suggested that adrenaline was without action on, or slightly relaxed the isolated sphincter of Oddi. Magee (1946) showed that adrenaline contracted the isolated sphincter in contrast to its action on the surrounding duodenal musculature. More elaborate studies on the effects of sympathomimetics on

the isolated sphincter were done by Benzi, Crema & co-workers (Benzi & Crema, 1960 ; Benzi & Crema, 1961 ; Crema & Benzi, 1961 ; Crema, Benzi & Berté, 1962 ; Crema, Berté, Benzi & Frigo, 1963 ; Crema & Berté, 1963). These authors showed that the sphincter contracted on α-adrenoceptor stimulation, but they were very cautious in their conclusions about β-adrenoceptor activity, because their preparations had a rapidly decreasing sensitivity to isoprenaline (Crema & Berté, 1963).

Liedberg & Persson (1970) investigated adrenoceptors in the cat choledochoduodenal junction *in situ* and found that the sphincter was relaxed by isoprenaline and terbutaline, a selective β_2-adrenoceptor stimulating agent (Bergman, Persson & Wetterlin, 1969). Terbutaline was shown to have less influence on the heart and the duodenum than isoprenaline in doses where both amines were equipotent in relaxing the sphincter. It was thought of interest to study the functions of the adrenoceptors in an isolated sphincter preparation, which is sensitive to β-adrenoceptor stimulation and make a more controlled evaluation of the quantitative relation between the effects of terbutaline and isoprenaline.

Methods

Seventy adult cats of both sexes were fasted 24 h before the experiment. They were anaesthetized with pentobarbitone sodium (Abbott) and bled. While being bathed with Krebs solution, the sphincter of Oddi was dissected free from surrounding duodenal tissue. The anatomical description of cat sphincter of Oddi by Boyden (1957) was used as a dissection guide. Before freeing it from surrounding duodenum, the sphincter was catheterized both through the common bile duct and through the pancreatic duct. If some duodenal tissue was left attached to the sphincter this was discovered by decreased sensitivity to sympathomimetic agents. Careful redissection then yielded a sensitive sphincter. When the sphincter was used in perfusion experiments it was contrived so that all the perfusion fluid passed through the whole sphincter without leakage.

Spirally cut sphincter preparations

The sphincter was cut from where the common bile duct changes over to sphincter of Oddi. Each sphincter yielded one 2–3 mm broad, clockwise or counterclockwise, spirally cut preparation. In some experiments, the part of the common bile duct just above the sphincter was mounted longitudinally as a tube 1–2 cm long.

Longitudinally cut sphincter preparations

One sphincter yielded one or two longitudinal strips. The sphincter was cut longitudinally starting at different points in relation to the entrances of the pancreatic and common bile duct.

Intact sphincter preparations

The sphincter was used as depicted in Fig. 1, which shows the whole sphincter mounted so that longitudinal movements are recorded simultaneously with the resistance to flow through the sphincter. Krebs solution was continuously perfused through the sphincter at a rate of 0·05 ml/h (Perfusor B. Braun Melsungen 71100). In some experiments, the sphincter was not perfused, but mounted with threads leaving the sphincter lumen open to the bathing fluid.

Duodenal preparations

Just distal to the choledochoduodenal junction, a 3 cm long piece of duodenum was cut, rinsed, and mounted longitudinally in the bath. In some experiments, the longitudinal and circular muscle layers were separated and mounted as 2–3 cm long strips.

The chosen preparation was immediately mounted in an organ bath (25 ml) with Krebs solution maintained at 38° C and gassed with carbogen (95% O_2/5% CO_2). The load on the sphincter preparations and the separated muscle layers of the duodenum was 0·5–1·0 g. The piece of duodenum was loaded with 2–4 g. The tissue activity was recorded isometrically through Statham transducers (FT 03) on a Grass Polygraph model 7 PI. In some experiments, the isotonic activity of the duodenum was recorded with a lever magnifying 7 times the duodenal longitudinal movements on smoked paper.

The following drugs were used: (±)-terbutaline sulphate (AB Draco, Lund, Sweden), (±)-isoprenaline hydrochloride (Sigma Chemical Company, USA), (−)-noradrenaline bitartrate (Sigma Chemical Company, USA), (−)-adrenaline bitartrate (Sigma Chemical Company, USA), tyramine hydrochloride (Light & Co. Ltd., England), propranolol hydrochloride (ICI Ltd., England), phenoxybenzamine hydrochloride (Smith, Kline & French, England), reserpine (CIBA, Schweiz), acetylcholine chloride (Calbiochem, USA), histamine hydrochloride, E. Merck, A.G.,

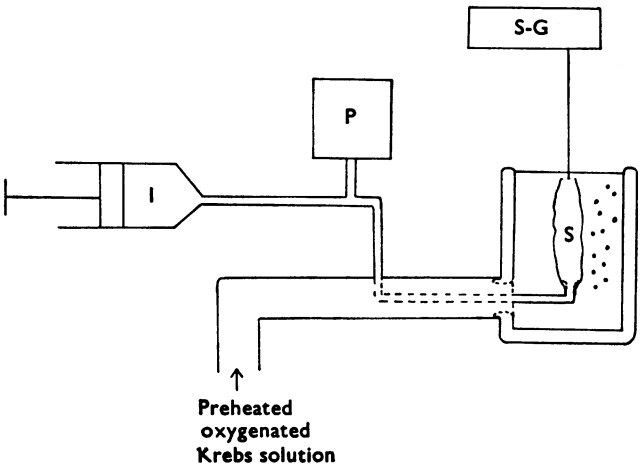
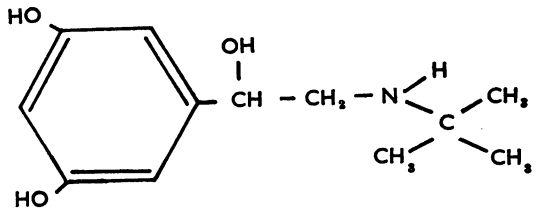


FIG. 1. Experimental arrangement for simultaneous recording of longitudinal movements and resistance to flow through the sphincter. I, Infusion apparatus; P, pressure transducer; S-G, strain-gauge transducer; S, sphincter of Oddi.



Terbutaline

FIG. 2. Structure of terbutaline.

Germany), pilocarpine hydrochloride (Pharmacopea Nordica), cholecystokinin (Prof. J. E. Jorpes, GIH Laboratories, Karolinska Inst., Stockholm), atropine sulphate (Pharmacopoeia Nordica), hexamethonium bromide (May & Baker Ltd., England) and tetrodotoxin (Sankyo, Japan). The structure of terbutaline (1-(3·5-dihydroxyphenyl)-2-(t-butylamino)-ethanol is shown in Fig. 2. The drugs were dissolved in fresh glass-distilled water before each experiment. In the case of isoprenaline the solution was stabilized with ascorbic acid 0·2 mg/ml. The preparations were allowed a stabilization period of at least 30 min before the experiment started. Drugs were added to the bathing solution and were allowed to act for 2–5 minutes. Exceptions to this rule were the blocking agents dibenzyline, propranolol, atropine, and hexamethonium, which were allowed to act for up to 15 minutes.

Results

Common bile duct preparation

All five preparations from the common bile duct contracted to adrenaline ($4{\cdot}8\times10^{-7}$M), noradrenaline ($5{\cdot}0\times10^{-7}$M–$1{\cdot}0\times10^{-6}$M), and acetylcholine ($7{\cdot}3\times10^{-7}$M). Three of these preparations from the common bile duct showed spontaneous activity, which was relaxed by terbutaline ($1{\cdot}8\times10^{-5}$M) and isoprenaline ($9{\cdot}5\times10^{-7}$M).

Spirally and longitudinally cut sphincter preparations

Four spirally cut sphincter preparations contracted to adrenaline ($4{\cdot}8\times10^{-7}$M to $9{\cdot}6\times10^{-7}$M) and noradrenaline ($5{\cdot}0\times10^{-7}$M to $1{\cdot}0\times10^{-6}$M), to acetylcholine ($7{\cdot}3\times10^{-7}$M) and to histamine ($1{\cdot}1\times10^{-6}$M) but isoprenaline in concentrations up to $9{\cdot}5\times10^{-6}$M and terbutaline up to $3{\cdot}5\times10^{-5}$M had no effect on the unstimulated preparation. In three experiments, pilocarpine ($2{\cdot}0\times10^{-5}$M) was mixed with Krebs solution. This treatment, however, did not make the preparation sensitive to β-adrenoceptor stimulating agents,

All twenty longitudinal strips showed at least the same sensitivity as the spirally cut preparations: eight strips showed regular spontaneous activity for more than 3 h ; these strips showed sensitivity to β-adrenoceptor stimulating agents. Isoprenaline ($1{\cdot}9\times10^{-8}$M to $9{\cdot}5\times10^{-6}$M) and terbutaline ($1{\cdot}8\times10^{-7}$M to $3{\cdot}5\times10^{-5}$M) inhibited the spontaneous contractions of the sphincter, and this effect was blocked by propranolol ($3{\cdot}9\times10^{-7}$M). Isoprenaline was between 2–18 times as active as terbutaline when comparing doses which were equipotent in inhibiting the sphincter. Cutting the sphincter longitudinally by starting at different points in relation to the entrances of the pancreatic and common bile ducts did not seem to be of any importance for the sensitivity of the preparation.

Intact sphincter preparations

The intact perfused sphincter showed rhythmic spontaneous activity similar to the longitudinal strips in thirty-three out of forty-one sphincters. In eighteen preparations, this activity was suitable for testing both α- and β-adrenoceptor stimulating agents for more than 3 hours. When the activity had ceased it could be restored by excitatory agents, but not by changing the load on the preparation. The spontaneous longitudinal contractions and the increased passage pressure through the sphincter were recorded simultaneously. The two most usual patterns of activity

(spikes of contractions 3–7/min from a basal level, mixed with periods of increased activity 0·5–1 min in duration) are shown in Fig. 3. This parallelism between the longitudinal movements and the passage pressure was not changed by any change in frequency of contractions. An increase in longitudinal contractions and activity caused by noradrenaline, adrenaline, tyramine, acetylcholine, or histamine was simultaneously shown as a corresponding rise in passage pressure. Similarly, none of the relaxing agents isoprenaline, terbutaline, or cholecystokinin could separate the two effects. When the preparations were stretched mechanically in order to simulate the longitudinal movements of the sphincter, no change in propulsive pressure was recorded.

In the following quantitative report about effects on spontaneously active intact sphincter both perfused and non-perfused intact sphincters are included.

One sphincter showed slight relaxation to noradrenaline ($5{\cdot}0 \times 10^{-7}$M to $1{\cdot}0 \times 10^{-7}$M). All the other sphincters contracted and increased their activity when adrenaline ($6{\cdot}0 \times 10^{-7}$M to $9{\cdot}6 \times 10^{-7}$M), noradrenaline ($2{\cdot}5 \times 10^{-7}$M to $1{\cdot}0 \times 10^{-6}$M) and tyramine ($2{\cdot}3 \times 10^{-6}$M to $9{\cdot}2 \times 10^{-6}$M) were added to the bath (Fig. 4). These effects were blocked by phenoxybenzamine ($1{\cdot}7 \times 10^{-7}$M). After α-adrenoceptor blockade with phenoxybenzamine, a reversed effect, that is a relaxation, was seen in those preparations which still had a spontaneous activity (Fig. 5). Phenoxybenzamine in doses of about $1{\cdot}7 \times 10^{-5}$M abolished the spontaneous contractions of the sphincter.

The spontaneously active sphincters relaxed when isoprenaline ($3{\cdot}8 \times 10^{-8}$M to $7{\cdot}6 \times 10^{-7}$M), terbutaline ($1{\cdot}8 \times 10^{-7}$M to $1{\cdot}4 \times 10^{-5}$M), or cholecystokinin (0·004–0·032 i.u./ml) (Fig. 6) was added to the bath. Threshold activity of the relaxing agents was recorded as a decrease in frequency of contractions or a diminished amplitude of the contractions or both events simultaneously. In about one-third of the preparations these agents caused a small decrease in basal tension. This decrease showed variation on repetition and was not used as basis for quantitative evaluation.

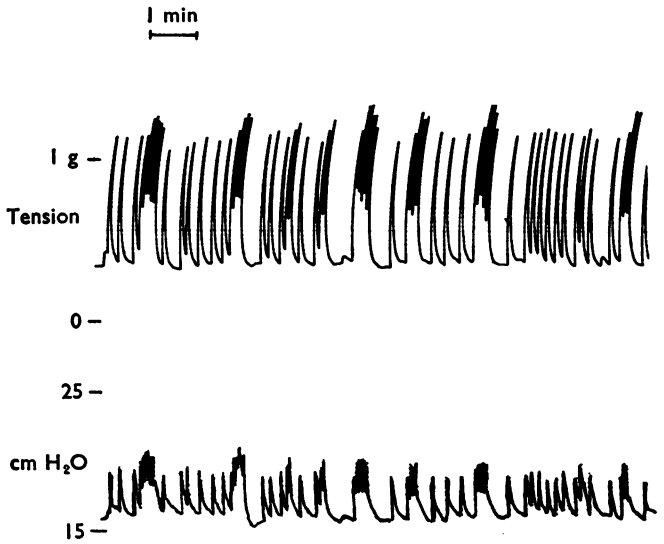


FIG. 3. Isolated perfused sphincter of Oddi. Upper trace, two patterns of spontaneous activity recorded as longitudinal sphincter movements. Lower trace, the simultaneous variations in resistance to flow through the sphincter.

The effects of isoprenaline and terbutaline were abolished by propranolol ($3{\cdot}9 \times 10^{-7}$M) (Fig. 7) which, however, did not affect the action of cholecystokinin. Propranolol ($3{\cdot}9 \times 10^{-7}$M to $3{\cdot}9 \times 10^{-6}$M) was without effect on the sphincter activity but in one case it repeatedly caused slightly increased tension of the sphincter. In ten sphincter preparations, the doses of isoprenaline and of terbutaline, sufficient to inhibit the spontaneous contractions of the sphincter, were determined. Initially, doses were added cumulatively to the bath in a geometric progression, each new dose doubling the concentration of the drug in the bath. Thus a fairly good idea was obtained of the doses needed to abolish the activity. Abolition of activity was achieved by varying doses of the β-adrenoceptor stimulating agents during the experiment, but the ratio between activity of isoprenaline and of terbutaline seemed to be constant for the same preparation. Isoprenaline was 5–18 times as active as

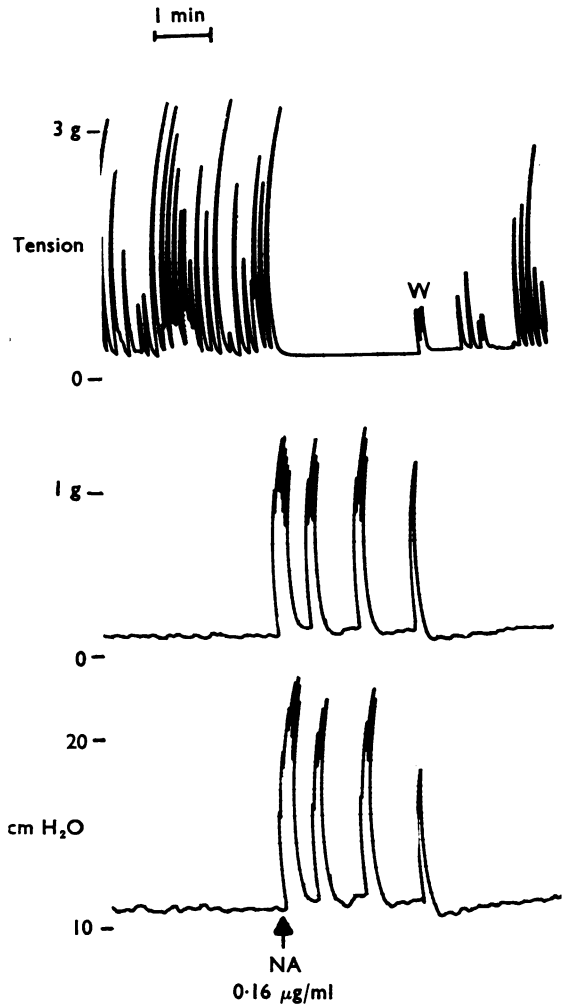


FIG. 4. Isolated perfused sphincter of Oddi. Upper trace, longitudinal movements of duodenum are extinguished by noradrenaline (NA). Middle trace, noradrenaline induces rhythmic contractions as shown by longitudinal activity of the sphincter. Lowest trace, noradrenaline induces increase in resistance to flow through the sphincter. W=wash.

terbutaline on a molar basis. If the relaxing drug was not rinsed out, the spontaneous activity of the sphincter gradually returned.

No decrease in the sphincter activity was seen either when phenoxybenzamine ($1{\cdot}7 \times 10^{-8}$M to $1{\cdot}7 \times 10^{-7}$M), atropine ($1{\cdot}4 \times 10^{-6}$M to $5{\cdot}8 \times 10^{-6}$M (Fig. 8), hexamethonium ($1{\cdot}4 \times 10^{-5}$M to $1{\cdot}1 \times 10^{-4}$M), tetrodotoxin (1 μg/ml) was added to the bath. The longitudinal and intact sphincter preparation where the spontaneous activity had ceased or had not been recorded, were tested for stable response to acetylcholine or noradrenaline. These agents contracted the sphincter and also induced a rhythmic activity (see Fig. 4). The effect of β-adrenoceptor agonists was evaluated by adding them to the bath 30 s before the addition of acetylcholine or noradrenaline. In some experiments, acetylcholine induced a relatively constant level of contraction. Then the effect of β-adrenoceptor agonists was tested after the preparation had reached this level. The effect was measured between two control doses of the excitatory agent. Of twenty-seven preparations treated in this way, only fourteen were relaxed by the β-adrenoceptor agonists. The responses were difficult to reproduce in the same preparation because of varying sensitivity to the excitatory, and probably also to the inhibitory, agents.

In five cats treated with reserpine (3 mg/kg) 16 h before the experiments, no effect of tyramine in concentrations up to $4{\cdot}6 \times 10^{-5}$M was obtained. However, the

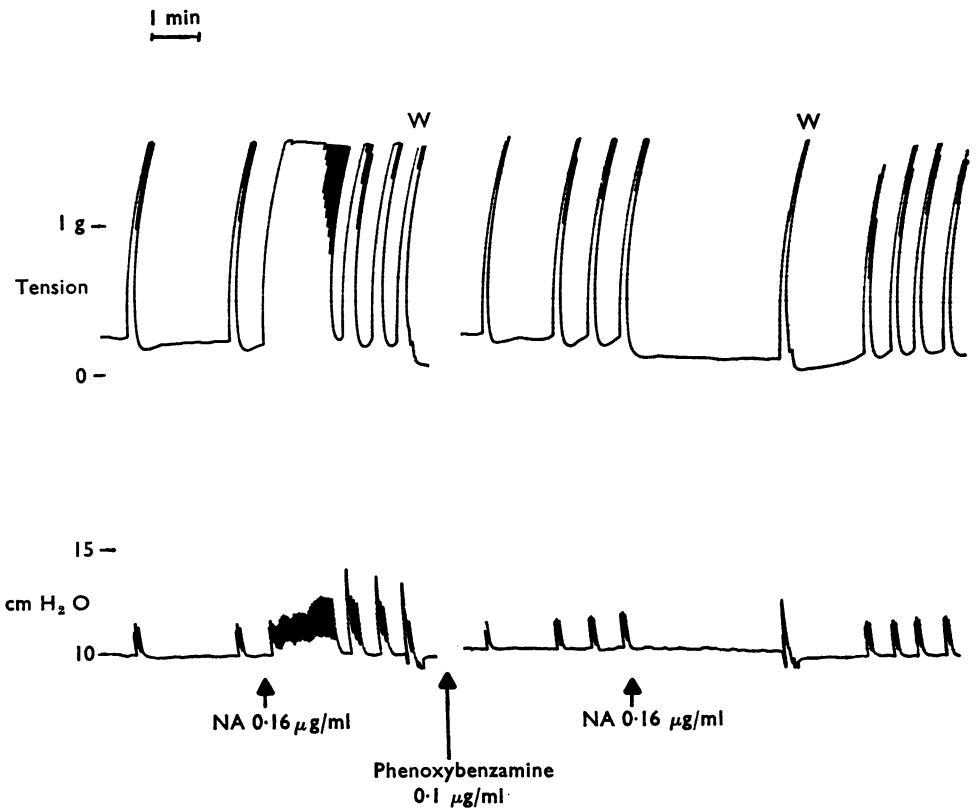


FIG. 5. Isolated perfused sphincter of Oddi. Upper traces, longitudinal sphincter movements. Lower traces, resistance to flow through the sphincter. Left, noradrenaline induced contraction and increased propulsive pressure. Right, inhibition of the action of the same dose of noradrenaline 10 min after the addition of phenoxybenzamine to the bath. W = wash.

excitatory effect of noradrenaline was potentiated in the presence of tyramine as shown in Fig. 9. The sphincters from the reserpinized cats had a spontaneous activity similar to the normal ones and showed the same reactions to the adrenoceptor stimulating agents.

The spontaneous rhythmic activity of the intact sphincters increased the resistance to flow through the sphincters. When the perfusion tube was disconnected from the infusion apparatus and held so that the perfusion pressure was constant (10–20 cm H_2O), the spontaneous activity of the sphincter was insufficient to decrease the fluid level in the perfusion tube.

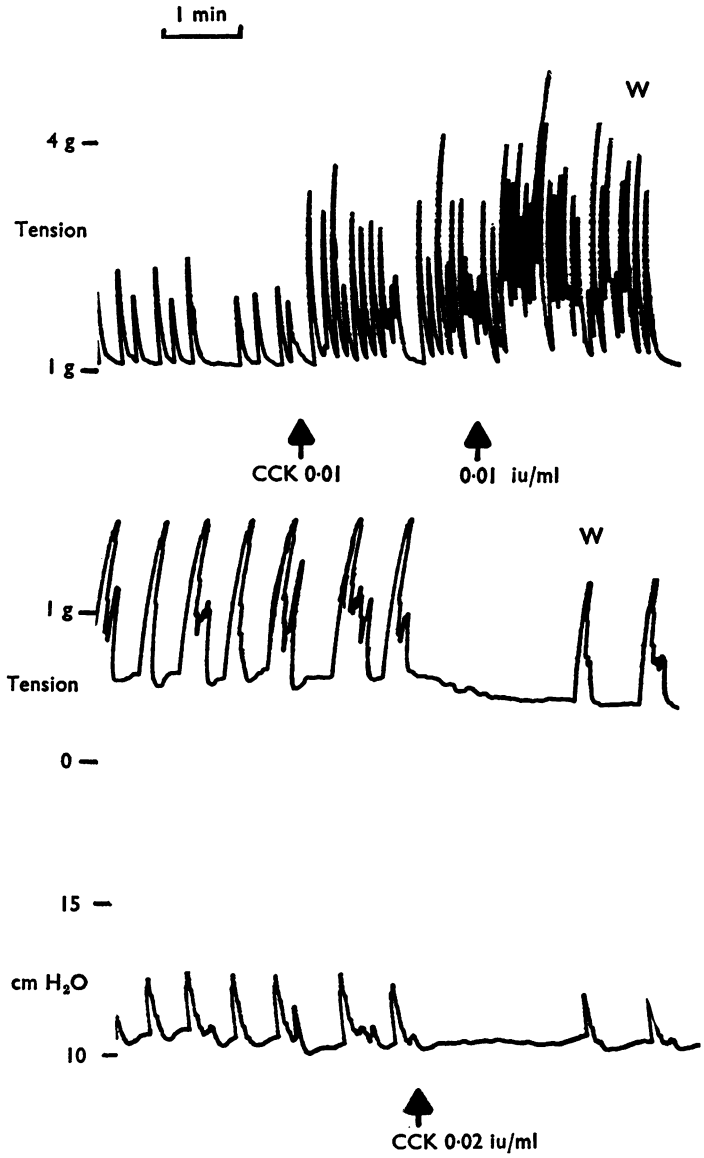


FIG. 6. Upper trace, isolated duodenum. Middle & lower traces as in Fig. 3 isolated perfused sphincter of Oddi. Cholecystokinin (CCK) excites the duodenum and relaxes the sphincter. W=wash.

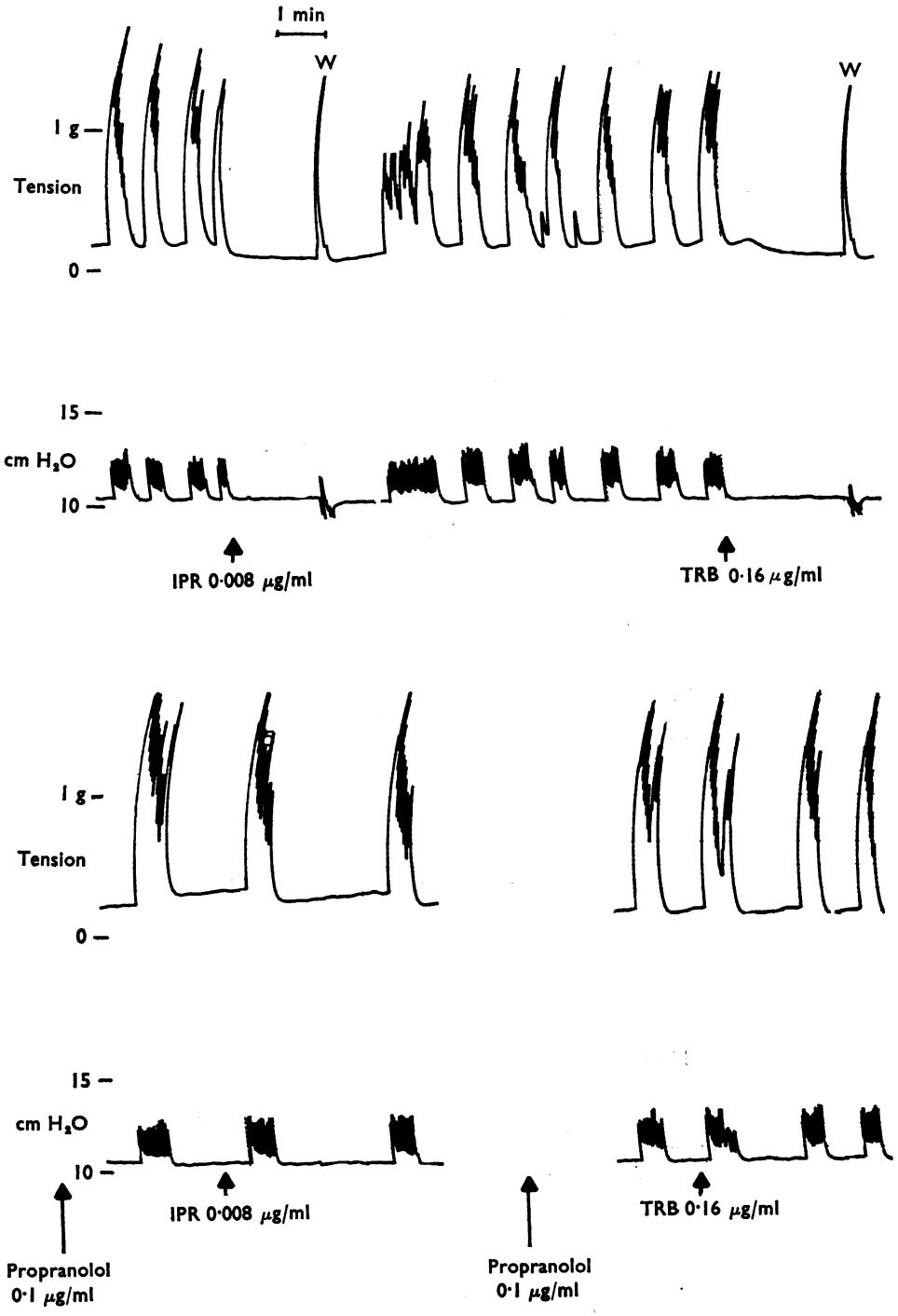


FIG. 7. Isolated perfused sphincter of Oddi. Upper traces, as in Fig. 3. Inhibitory actions of isoprenaline (IPR) and terbutaline (TRB) on the sphincter. Lower traces, as in Fig. 3. Propranolol was added to the bath 10 min before the addition of isoprenaline and terbutaline. The inhibitory actions of these agents was blocked by propranolol. W=wash.

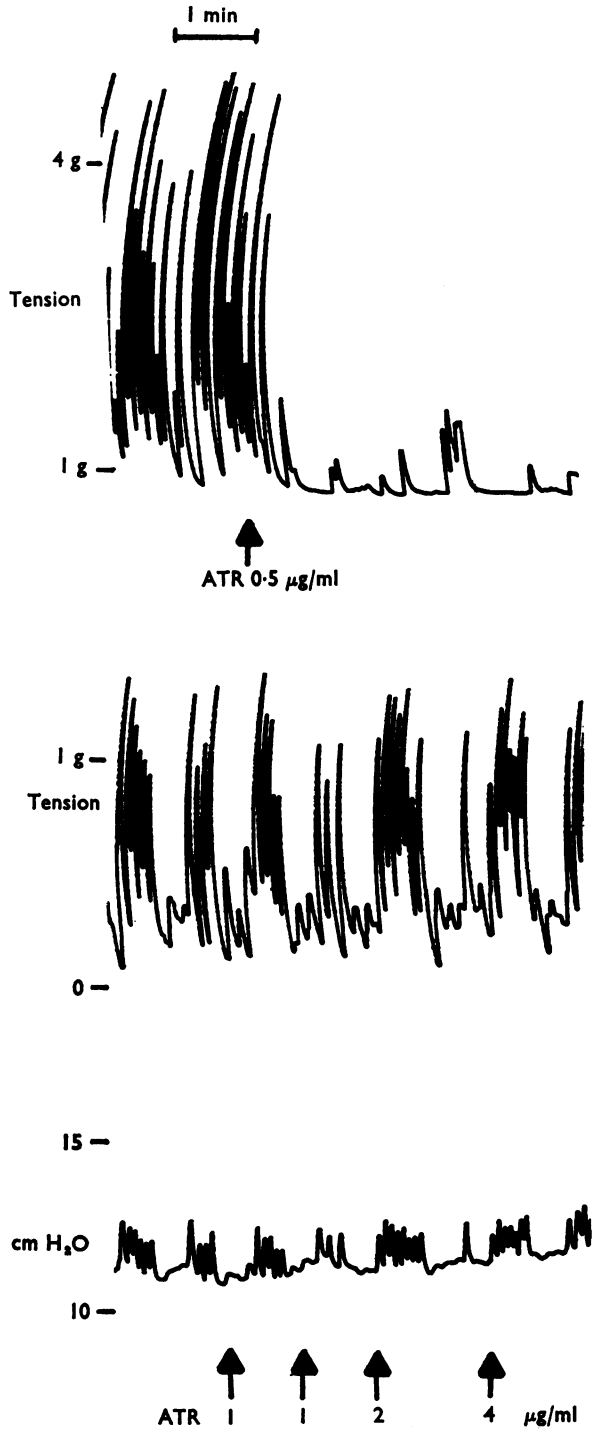


FIG. 8. Isolated duodenum and isolated perfused sphincter of Oddi. Traces as in Fig. 4. Atropine (ATR) abolishes the duodenal movements but leaves the sphincter unaffected.

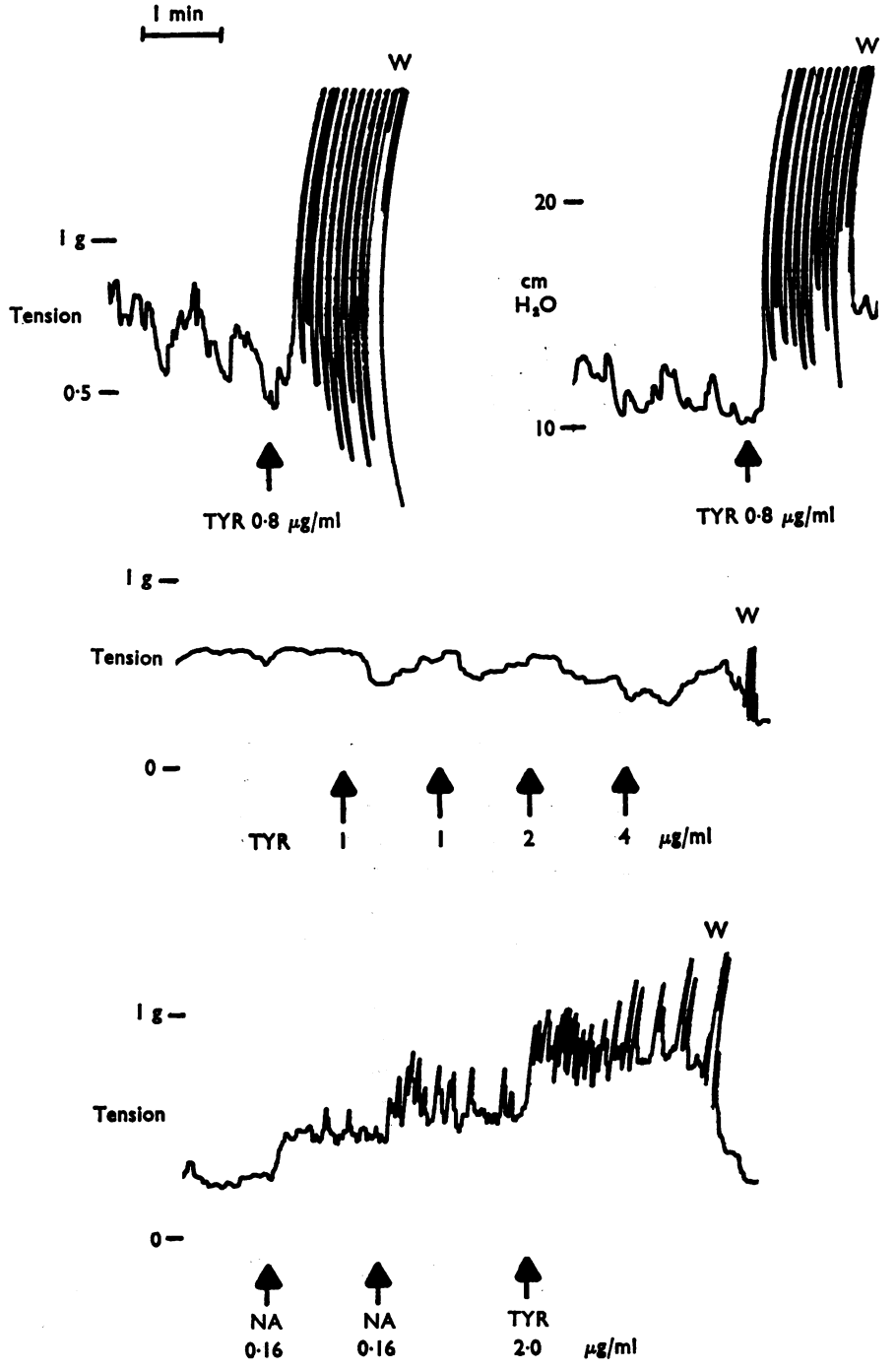


FIG. 9. Isolated perfused sphincter of Oddi. Upper traces, left: longitudinal movements; right: simultaneous variations in propulsive pressure through the sphincter from non-reserpinized cat. Tyramine (TYR) excited the sphincter. Middle trace, tyramine was without effect on the sphincter from a reserpinized cat (longitudinal movements recorded). Lowest trace, effect of noradrenaline on sphincter from reserpinized cat. When tyramine was added to the noradrenaline in the bath, the effect of the noradrenaline was potentiated. W=wash.

Duodenal preparations

Noradrenaline ($5{\cdot}0 \times 10^{-7}$M to $1{\cdot}0 \times 10^{-6}$M) and adrenaline ($4{\cdot}8 \times 10^{-7}$M to $9{\cdot}6 \times 10^{-7}$M) relaxed the duodenal preparations (Fig. 4). This relaxation was blocked by phenoxybenzamine ($1{\cdot}7 \times 10^{-6}$M) plus propranolol ($3{\cdot}9 \times 10^{-6}$M). Propranolol ($1{\cdot}9 \times 10^{-5}$M to $3{\cdot}9 \times 10^{-5}$M) relaxed the duodenum. Of the separated muscle layers (five cats), the longitudinal strips showed spontaneous activity, which was inhibited by the α- and β-adrenoceptor agonists. The unstimulated strip of the circular muscle layers was not affected by the sympathomimetic agents.

Isoprenaline and terbutaline relaxed the duodenum and inhibited the spontaneous activity. This effect was blocked by propranolol ($3{\cdot}9 \times 10^{-7}$M). No acceptable dose-response relations were obtained, either in the experiments recording isometric contractions or in the isotonic preparations. A quantitative comparison between the activity of isoprenaline and terbutaline on duodenum, made in the same way as with the sphincters, showed isoprenaline to be 35–90 times as active as terbutaline (ten preparations).

Discussion

The investigation has shown that different preparations of the sphincter of Oddi show different sensitivity to β-adrenoceptor stimulation. The effect obtained with α-adrenoceptor agonists is easier to achieve. Both longitudinal and intact sphincter preparations were suitable for evaluation of β-adrenoceptor agonists. This is not in agreement with the findings of Crema & Berté (1963) who reported a rapid decrease in sensitivity to isoprenaline for their isolated cat sphincter preparations. The sphincter of Oddi is embedded in duodenal tissue, but the pharmacological results indicate that the sphincter can be dissected free from the duodenum, since α-adrenoceptor agonists excite the sphincter and relax the duodenum ; cholecystokinin on the contrary, relaxes the sphincter and stimulates the duodenum, and atropine has no effect on sphincter activity but decreases the duodenal movements.

The results from spirally cut sphincter preparations, being sensitive to α-adrenoceptor but not to β-adrenoceptor agonists, agree with the findings of Crema and co-workers (Benzi & Crema, 1961 ; Crema & Benzi, 1961 ; Crema & Berté, 1963) in preparations of this kind. The complexity of the muscle pattern in the cat choledochoduodenal junction (Boyden, 1957) makes it difficult to cut the sphincter without doing considerable damage to the musculature. This helps to explain the difficulties experienced except with an intact sphincter preparation which showed rhythmic spontaneous activity.

The spontaneous activity of the sphincters was not inhibited by atropine, phenoxybenzamine, hexamethonium, or tetrodotoxin. The activity was also present in sphincters from reserpinized cats. Therefore it is unlikely that the automaticity of the sphincters is produced by release of a nervous transmitter. In this respect, the sphincter is comparable in behaviour to many other visceral smooth muscles which show myogenic spontaneous activity (reviewed by Holman, 1968). Phenoxybenzamine ($1{\cdot}7 \times 10^{-5}$M) did inhibit the contractions of the sphincter, but this is probably a non-specific effect of the high dose.

The frequency and appearance of recorded contractions were similar in intact, isolated sphincters, and in those remaining *in situ*, especially when the cats had an atonic duodenum (Liedberg & Persson, 1970).

Various opinions have been expressed about the role of the sphincter in the transport of bile into the duodenum. Watts & Dunphy (1966) think that the sphincter acts as a pump activated by cholecystokinin but get no support for this suggestion from Wyatt (1968) or Hedner & Rorsman (1969). These authors have reached their conclusions from studies on pressure differences between common bile duct and duodenum. In my experiments on isolated intact sphincter, the sphincter activity has no propulsive function. Moreover, its activity inhibits the flow, and the activity is abolished by cholecystokinin. As the *in vitro* recorded activity is similar to that found *in vivo* (Liedberg & Persson, 1970) it can be presumed that the sphincter function too is similar *in vitro* and *in vivo*. This would also mean that the relaxing effect produced by β-adrenoceptor stimulating agents facilitates the passage of bile into the duodenum.

Crema *et al.* (1963) in their intact sphincter preparations *in vitro* did not report a spontaneous rhythmic activity that could be inhibited by isoprenaline. None the less, these authors reported spontaneous contractions which stopped the flow through the sphincter (Crema & Berté, 1963). Their report agrees with the results presented here on the similarity of time course of longitudinal activity and resistance to flow through the sphincter.

The results from experiments *in situ* concerning functions of α- and β-adrenoceptors of cat choledochoduodenal junction (Liedberg & Persson, 1970) are confirmed by this *in vitro* study, which shows that α-adrenoceptors excite the sphincter and β-adrenoceptors inhibit it and decrease flow resistance.

The part of the common bile duct close to the sphincter probably contains smooth muscles with adrenoceptors similar to the sphincter, as it contracted to noradrenaline and adrenaline and relaxed to β-adrenoceptor agonists. Ludwick (1966) studied the actions of drugs on isolated common bile ducts from man, monkey, and dog. He found that noradrenaline was excitatory but used very high doses. This means that for isoprenaline its α-adrenoceptor stimulating activity might mask the inhibitory effect of the β-adrenoceptor stimulation (Sullivan & Marshall, 1970) and that atropine probably acts as a non-specific depressant of spontaneous movements of the common bile duct.

The inability of the β-adrenoceptor antagonist propranolol to affect the response to cholecystokinin *in situ* is confirmed by this study *in vitro*. The finding that the β-adrenoceptor antagonist, propranolol, in higher doses, inhibited duodenal longitudinal movements agrees with the report by Mylecharane & Raper (1970) who discuss the possibility that a similar effect on rabbit ileum is a non-specific action of propranolol. This action of propranolol was not found on the sphincter and thus adds to the evidence of differences between duodenal and sphincter smooth muscle responses. Actually, one sphincter responded with increased tension to propranolol.

It was difficult to make a quantitative estimate of the dose-response for the β-adrenoceptor agonist on the isolated sphincter. Neither the strength of contractions nor the frequency of contraction spikes offered a suitable basis for such an estimation. β-Adrenoceptor stimulation irregularly affected both these parameters and sometimes caused a simultaneous fall in basal tonus. The difficulties experienced here are comparable to those described for the isolated uterus where, however, an evaluation of inhibitory sympathomimetic agents on a preparation stimulated with carbachol has been successful (Hawkins, 1964). This procedure was not suitable here because the response of the sphincter to stimulant agents was too

inconsistent. Instead, this preparation offers a possibility of determining the activity of β-adrenoceptor agonists on the sphincter of Oddi without the influence of other agents, that is evaluation on the spontaneously active sphincter. The choice of making a quantitative comparison between isoprenaline and terbutaline on a level at which both agents were just able to extinguish the automaticity of the sphincter seemed justified. The doses needed for this effect were well within the range for submaximal β-adrenoceptor stimulating effects on other isolated organs (Persson & Olsson, 1970).

Isoprenaline and terbutaline had no effect on the sphincter after β-adrenoceptor blockade indicating that within the dose range used these agents did not stimulate α-adrenoceptors. Isoprenaline (in submaximal effective doses) is not taken up in nerve terminals or muscle tissue in rabbit hearts (Andeń, Corrodi, Ettles, Gustafsson & Persson, 1964). Persson (1971) using the same technique as Andeń *et al.* (1964) has shown that terbutaline is unable to restore the tyramine response in hearts from reserpinized rabbits, indicating that this compound like isoprenaline is not actively taken up into nerve terminals. These findings favour the possibility that differences in potency at the β-adrenoceptor level yield the quantitative relation between the effects of isoprenaline and terbutaline.

The pharmacology of terbutaline has been investigated by Persson & Olsson (1970) and Persson & Johnson (1970) who showed it to be selectively active on bronchial muscle, that is on the β_2-adrenoceptor according to Lands' classification (Lands, Groblewski & Brown jun., 1966 ; Lands, Luduena & Buzzo, 1967). They found isoprenaline to be about 18 times as active as terbutaline on the isolated guinea-pig bronchial preparation. The activity ratio between these compounds found both in the longitudinal strips of the sphincter of Oddi and in the intact sphincter is of similar magnitude. On the isolated guinea-pig heart Persson & Olsson (1970) report isoprenaline to be more than 500 times as active (chronotropic and inotropic activity) as terbutaline.

The results obtained with tyramine on sphincters from normal and reserpinized cats indicate the presence of adrenergic nerves in the cat sphincter of Oddi. This view is supported by histological studies on adrenergic innervation of the cat choledochoduodenal tract (unpublished observations of P. Alm, G. Liedberg, C. Owman & N. O. Sjöberg). These investigators found that the adrenergic nerve terminals in the longitudinal musculature of the duodenum accumulate in the sphincter region.

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